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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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10/529,352

01/11/2006

Nigel Tooke

21465-523 NATL

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06/25/2008

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EXAMINER

WOOLWINE, SAMUEL C

ART UNIT

PAPER NUMBER

1637

MAIL DATE

DELIVERY MODE

06/25/2008

PAPER

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

<b>Office Action Summary</b>	<b>Application No.</b> 10/529,352	<b>Applicant(s)</b> TOOKE, NIGEL	
	<b>Examiner</b> SAMUEL WOOLWINE	<b>Art Unit</b> 1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

### Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

### Status

- 1) ☒ Responsive to communication(s) filed on 12 February 2008.
- 2a) ☒ This action is **FINAL**.                      2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

### Disposition of Claims

- 4) ☒ Claim(s) 1-7,9-21 and 23-35 is/are pending in the application.
- 4a) Of the above claim(s) 9-11 and 27-35 is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 1-7,12-21 and 23-26 is/are rejected.
- 7) ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

### Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on \_\_\_\_\_ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.  
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).  
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

### Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All    b) ☐ Some \*    c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
  2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
  3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\* See the attached detailed Office action for a list of the certified copies not received.

### Attachment(s)

- |  |   |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892)          | 4) <input type="checkbox"/> Interview Summary (PTO-413)           |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date. _____                                      |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08)          | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date _____  | 6) <input type="checkbox"/> Other: _____                          |

## DETAILED ACTION

### *Status*

Applicant's reply filed 02/12/2008 is acknowledged. Claims 1-7, 9-21, 23-35 are pending. Claims 9-11 and 27-35 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 09/25/2007.

The rejection of claims 19 and 21 under 35 U.S.C. 112, 1<sup>st</sup> paragraph is maintained for the reasons of record and reiterated below. Applicant's remarks will be addressed following the rejection.

The rejection of claims 1-8, 12-18, 22, 23, 25 and 26 under 35 U.S.C. 103(a) over Nyren (US 6,210,891) in view of Melamede (US 4,863,849), Kotewicz (US 5,244,797) and Inouye (US 5,434,070) is withdrawn in view of Applicant's amendment to claim 1, as none of these references teach a RNA-secondary structure reducing reagent. Consequently, the rejections of claims 20 and 24 based upon this combination of references in view of Myers (PNAS 77(3):1316-1320, March 1980) and in view of Malek (US 5,665,545), respectively, are also withdrawn. New grounds of rejection are set forth below. Therefore, Applicant's arguments with respect to these rejections are moot.

### ***Claim Rejections - 35 USC § 112***

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 21 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. This is a new rejection necessitated by amendment.

Claim 21 has been amended to recite *wherein the alpha-S-ATP is incorporated into the extended primer*. However, as claim 1 recites that the RNA dependent polymerase is a reverse transcriptase, which is an enzyme that polymerizes dNTPs, not NTPs, this limitation does not make practical sense. It is presumed such a limitation would correspond to an alternative embodiment wherein the RNA dependent polymerase is an RNA dependent RNA polymerase (e.g. paragraph [0056] of the published application).

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 19 and 21 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. These claims recite that the primer comprises dATP (claim 19) or ATP (claim 21), wherein the dATP or ATP is exchanged for, respectively, the alpha-S analog. These claims therefore appear to recite primers comprising these analogs from the beginning (rather than as a result of the primer

extension process). There is no support in the original disclosure for such subject matter. This is a NEW MATTER rejection. Applicant may overcome this rejection by pointing out support in the original disclosure that clearly indicates Applicant conceived of a primer comprising these analogs.

### ***Response to Arguments***

Applicant's arguments filed 02/12/2008 have been fully considered but they are not persuasive. The claims still read on the situation where the primers comprised the alpha-S analog from the beginning, which was a condition not disclosed in the original disclosure. The only "exchange" for which there is support is the exchange of dATP or ATP with the alpha-S analog in the extension reaction (paragraphs [0074]-[0076] in the published instant application). Applicant is advised to amend claims 19 and 21 to refer to the primer extension reaction, rather than the primer. For example: "The method of claim xyz, wherein dATP is replaced with alpha-S-dATP, wherein the alpha-S-dATP is incorporated into the extended primer."

### ***Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 1-7, 12, 14-19 and 25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ronaghi (WO 00/43540) in view of Kotewicz et al (US 5,244,797,

prior art of record) and Inouye (US 5,434,070, prior art of record). This is a new rejection necessitated by amendment.

Ronaghi teaches a method of sequencing DNA and RNA by pyrophosphate sequencing (i.e. "pyrosequencing"). Ronaghi teaches that

With regard to claim 1, Ronaghi teaches *a method for determining the identity of at least one nucleotide in a RNA-molecule* (page 1, first paragraph: "The present invention relates to methods of nucleic acid sequencing..."; page 5, first paragraph: "The nucleic acid may be DNA or RNA...") *comprising the steps of:*

*(a) providing a single stranded form of the RNA-molecule* (See page 4, last full paragraph: "the present invention thus provides a method of identifying a base at a target position in a sample nucleic acid sequence wherein a primer, which hybridises to the sample nucleic acid immediately adjacent to the target position, is provided and the sample nucleic acid and primer are subjected to a polymerase reaction". To provide the template in single-stranded form would have been obvious from the statement on page 5, first full paragraph: "The DNA may also be single or double-stranded—whilst a single-stranded DNA template has traditionally been used in sequencing reactions, or indeed in any primer-extension reaction, it is possible to use a double-stranded template; strand displacement, or a localised opening-up of the two DNA strands may take place to allow primer hybridisation and polymerase action to occur." Thus, since Ronaghi teaches RNA templates as well (e.g. page 5, first paragraph and last full paragraph), it would have been obvious to one of ordinary skill to provide the RNA template in single-

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stranded form, were it not already so, "to allow primer hybridisation and polymerase action to occur".);

*(b) hybridising an oligonucleotide primer binding to a predetermined position of the RNA molecule* (page 4, last full paragraph: "wherein a primer, which hybridises to the sample nucleic acid immediately adjacent to the target position, is provided");

*(c) performing at least one primer extension reaction in an extension reaction solution comprising reagents to detect light triggered by the release of PPi* (See page 8, beginning at third full paragraph: "...methods which rely on monitoring the release of inorganic pyrophosphate (PPi) are particularly preferred. In this case, incorporation of the nucleotide will be measured indirectly by enzymatic detection of released PPi." See also page 8, last paragraph and entire page 9, where Ronaghi teaches luciferin, luciferase, ATP sulphurylase, which are reagents to detect light triggered by the release of PPi.)

*and a RNA-secondary structure reducing reagent* (See page 4, last full paragraph: "the present invention thus provides a method...characterized in that, a single-stranded nucleic acid binding protein is included in the polymerase reaction step." See also page 7, first full paragraph, where Ronaghi lists single-stranded nucleic acid binding proteins including T4 gene 32 protein and SSB. Ronaghi also teaches DMSO and formamide (top of page 16). Each of these is specifically recited in Applicant's specification as exemplary RNA-secondary structure reducing reagents.),

*whereby the oligonucleotide primer is extended on the RNA-molecule through incorporation of at least one nucleotide by the action of a RNA dependent polymerase,*

*whereby the polymerase is a reverse transcriptase* (page 5, last full paragraph: “In the case of a RNA template, such a polymerase enzyme may be a reverse transcriptase enzyme.”);

*(d) detecting the presence or absence of incorporation, thereby indicating the nucleotide identity of the RNA molecule in the relevant position* (page 6, last paragraph: “detecting the presence or absence of incorporation”);

*whereby step (c) to (d) optionally are repeated* (page 6, last paragraph: “Hence, a sequencing protocol may involve annealing a primer as described above, performing a polymerase-catalysed primer extension step, detecting the presence or absence of incorporation, and repeating the nucleotide addition and primer extension steps etc. one or more times.”).

With regard to claim 2, see last statement regarding claim 1.

With regard to claim 4, Ronaghi teaches released PPi, which is a detectable moiety, to indicate the presence or incorporation (page 8, third full paragraph: “incorporation of the nucleotide will be measured indirectly by enzymatic detection of released PPi”).

With regard to claim 5, the PPi is neutralized or removed via conversion into ATP by ATP sulphurylase, which ATP is then hydrolyzed by luciferase to produce light (see reaction, page 9).

With regard to claims 6 and 7, PPi is a residue molecule released by the primer extension reaction (i.e. the incorporation of a nucleotide; see statement regarding claim 4).



With regard to claim 19, Ronaghi teaches dATP $\alpha$ S analog in place of dATP (page 13, last full paragraph).

With regard to claim 25, Ronaghi teaches at page 12, first paragraph: "When mRNA is the sample nucleic acid, it may be advantageous to submit the initial sample, e.g. a serum sample, to treatment with an immobilised polydT oligonucleotide in order to retrieve all mRNA via the terminal polyA sequences thereof. Alternatively, a specific oligonucleotide sequence may be used to retrieve the RNA via a specific RNA sequence." Note that claim 25 does not require that the immobilized oligonucleotide used to capture the RNA molecule to the solid support is also the oligonucleotide used as the sequencing primer.

Ronaghi does not teach that the hybridization is performed in the presence of at least one RNase-inhibiting agent, or that the reverse transcriptase to be used essentially lacks RNase H activity, as recited in claim 1.

Ronaghi does not teach "recording" as recited in claim 3.

Ronaghi does not teach the particular types of reverse transcriptases recited in claim 12.

Ronaghi does not teach a mixture of reverse transcriptases as recited in claim 13.

Ronaghi does not teach the temperature, pH, nucleotide concentration, or salt concentration limitations as recited in claims 14-17, respectively.

With regard to claim 18, while Ronaghi teaches DNA primers, he does not teach DNA primers in the context of an RNA template.

Kotewicz teaches a method for synthesizing cDNA from an RNA template using a reverse transcriptase.

With regard to claim 1, Kotewicz teaches a reverse transcriptase that essentially lacks RNase H activity (column 2, lines 49-51).

With regard to claim 12, Kotewicz's enzyme is a M-MuLV reverse transcriptase (column 11, lines 9-15; M-MLV is the same as M-MuLV).

With regard to claim 14, Kotewicz uses this enzyme to perform primer extension from an RNA template at 37°C (column 14, lines 17-18).

With regard to claim 15, Kotewicz uses this enzyme to perform primer extension from an RNA template at pH 8.3 (column 14, line 13).

With regard to claim 16, Kotewicz uses this enzyme to perform primer extension from an RNA template at a nucleotide concentration of 0.5 mM (column 14, lines 14-15).

With regard to claim 17, Kotewicz uses this enzyme to perform primer extension from an RNA template at salt (KCl) concentration of 75 mM (column 14, line 13).

With regard to claim 18, Kotewicz uses this enzyme to perform primer extension from an RNA template using a DNA primer (dT)<sub>12-18</sub> (column 14, lines 17-18).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention of the instant application was made to use the reverse transcriptase essentially lacking RNase H activity taught by Kotewicz for the purpose of sequencing an RNA molecule by the method of Ronaghi. One would have been motivated to do so, because Kotewicz teaches that RNase H activity is a major problem when using reverse transcriptase to synthesize cDNA (column 1, line 47 through column 2, line 5). cDNA

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synthesis is precisely what would occur in the embodiment of sequencing taught by Ronaghi at page 5, last full paragraph: "In the case of a RNA template, such a polymerase enzyme may be a reverse transcriptase enzyme." One of ordinary skill in the art would have realized that the same problem taught by Kotewicz with regard to synthesizing cDNA would also have existed in the RNA sequencing embodiment taught by Ronaghi, and would therefore have been motivated to use a reverse transcriptase lacking RNase H activity.

It would also have been obvious to use the conditions of temperature, pH, salt and nucleotide concentrations, and to use a DNA primer (thus meeting the limitations of claims 14-18), since Kotewicz had shown these to be appropriate conditions for synthesis of DNA from an RNA template using reverse transcriptase.

With regard to claim 1, Inouye teaches at column 12, line 66 through column 13, line 9 (emphasis provided):

"For background and protocols on synthesis of cDNA and reverse transcript, see Molecular Cloning: A Laboratory Manual ("Maniatis") pages 129-130 and 213-216 (incorporated herein by reference). If it is desired to separate any RNase activity when such is present, the protocols referred to in Maniatis in the Chapter on Synthesis of cDNA may be referred to (page 213). See also Marcus et al., J. Virol., 14, 853 (1974) and other references cited at page 213. Other protocols are known in the art, such as including in the reverse transcription reaction mixture an inhibitor of RNase, such as vanadyl-ribonucleoside complexes or RNasin."

It would have been *prima facie* obvious to one of ordinary skill in the art to use an RNase inhibitor when practicing the RNA sequencing method suggested by the combined teachings of Ronaghi and Kotewicz, since it was known in the art that RNA was susceptible to RNase degradation, and Inouye teaches it was known in the art, in the context of a reverse transcriptase reaction, to add inhibitors of RNase.

With regard to claim 3, neither Ronaghi nor Kotewicz nor Inouye expressly teaches or suggests recording the nucleotides incorporated. However, it would have been obvious to one of skill in the art to do so. Otherwise, one practicing the method would have had to commit to memory the sequence in which nucleotides were incorporated (which, incidentally, would still be “recording”). What would have been the purpose of sequencing an RNA molecule and *not* recording the results?

Claim 13 is rejected under 35 U.S.C. 103(a) as being unpatentable over Ronaghi (WO 00/43540) in view of Kotewicz et al (US 5,244,797, prior art of record) and Inouye (US 5,434,070, prior art of record) as applied to claims 1-7, 12, 14-19 and 25 above, and further in view of Wilkinson et al (GB2351559, published January 3, 2001).

The teachings of Ronaghi, Kotewicz and Inouye have been discussed. None of these references teaches using a mixture of RNA dependent polymerases.

Wilkinson teaches using a mixture of reverse transcriptases to increase the sensitivity and product yield of reverse transcription (see abstract and page 5, lines 7-10, for example).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the method suggested by the combined teachings of Ronaghi, Kotewicz and Inouye by using a mixture of reverse transcriptases as proposed by Wilkinson in order to achieve the improvement in sensitivity and yield taught by Wilkinson.

Claim 20 is rejected under 35 U.S.C. 103(a) as being unpatentable over Ronaghi (WO 00/43540) in view of Kotewicz et al (US 5,244,797, prior art of record) and Inouye (US 5,434,070, prior art of record) as applied to claims 1-7, 12, 14-19 and 25 above, and further in view of Myers et al (PNAS 77(3):1316-1320, March 1980, prior art of record).

The teachings of Ronaghi, Kotewicz and Inouye have been discussed. These references do not teach using an RNA primer.

Myers teaches that reverse transcriptases can use RNA as a primer (see title, for example).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention of the instant application was made to use either DNA or RNA as the primer when practicing the method for sequencing RNA suggested by the combined teachings of Ronaghi, Kotewicz and Inouye, since both were known in the prior art to be suitable for primer extension by reverse transcriptase. See MPEP 2144.07 regarding the selection of a known material based on its suitability for its intended purpose.

Claims 23 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ronaghi (WO 00/43540) in view of Kotewicz et al (US 5,244,797, prior art of record) and Inouye (US 5,434,070, prior art of record) as applied to claims 1-7, 12, 14-19 and 25 above, and further in view of Malek et al (US 5,665,545, prior art of record).

The teachings of Ronaghi, Kotewicz and Inouye have been discussed. These references do not teach using rITP in place of rGTP during amplification of the RNA.

Malek teaches a method of amplifying RNA called TRAM (terminal repeat amplification method) and teaches that substitution of rITP for rGTP in an RNA amplification product alleviates pausing of reverse transcriptase due to secondary structure (stem-loop formation) when using the RNA in a subsequent primer extension reaction (column 24, lines 21-45).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention of the instant application was made to use the known method of RNA amplification taught by Malek when practicing the method for sequencing RNA suggested by the combined teachings of Ronaghi, Kotewicz and Inouye, since Ronaghi teaches the desirability of amplifying the nucleic acid to be sequenced (page 10, lines 1-4). Furthermore, it would have been obvious to exchange rITP for rGTP to produce an RNA amplification product, as Malek shows that such a product reduces pausing of the reverse transcriptase during primer extension. Primer extension mediated by reverse transcriptase was what was taught by Ronaghi at page 5, last full paragraph: "In the case of a RNA template, such a polymerase enzyme may be a reverse transcriptase enzyme."

Claim 26 is rejected under 35 U.S.C. 103(a) as being unpatentable over Ronaghi (WO 00/43540) in view of Kotewicz et al (US 5,244,797, prior art of record) and Inouye (US 5,434,070, prior art of record) as applied to claims 1-7, 12, 14-19 and 25 above, and further in view of Rothberg et al (US 6,274,320).

The teachings of Ronaghi, Kotewicz and Inouye have been discussed. These references do not teach determining “a quantity” of the RNA molecule by measuring the intensity of the incorporation signal and comparing it to a reference.

Rothberg teaches regarding pyrosequencing (column 17, lines 45-55):

“Typically, the PPi-based detection is calibrated by the measurement of the light released following the addition of control nucleotides to the sequencing reaction mixture immediately after the addition of the sequencing primer. This allows for normalization of the reaction conditions. Incorporation of two or more identical nucleotides in succession is revealed by a corresponding increase in the amount of light released. Thus, a two-fold increase in released light relative to control nucleotides reveals the incorporation of two successive dNTPs into the extended primer.”

Hence, it would have been obvious to one of ordinary skill in the art at the time the invention was made to do the same as taught by Rothberg in the context of sequencing RNA by the method suggested by the combined teachings of Ronaghi, Kotewicz and Inouye, in order to determine how many successive identical nucleotides were incorporated. This would constitute “a quantity of the RNA molecule”, and furthermore the control nucleotides would constitute “a reference”.

***Conclusion***

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SAMUEL WOOLWINE whose telephone number is (571)272-1144. The examiner can normally be reached on Mon-Fri 9:00am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.



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Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Samuel Woolwine/  
Examiner, Art Unit 1637

/Young J Kim/  
Primary Examiner, Art Unit 1637